

VIEWPOINT

Antioxidants, Statins, and Atherosclerosis

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Research into the oxidation of lipoproteins has yielded many new insights into the pathogenesis of atherosclerosis. However, despite lipoprotein oxidation's biologically plausible role in atherogenesis, several studies have reported inconsistent effects of antioxidants on clinical coronary end points, in sharp contrast with the studies of lipid modification with the 3-hydroxy-3-methylglutaryl coenzyme A inhibitors, or statins. There appears to be little support for the use of antioxidants in coronary prevention. However, the picture remains incomplete. What are the limitations of available antioxidant studies and the agents used? Until the picture can be clarified, lipid modification with strategies proved to reduce the risk for coronary events, such as statins or dietary changes in the style of the Mediterranean diet, should be better implemented in clinical practice. (J Am Coll Cardiol 2003;41:1205-10)

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Research into the oxidation of lipoproteins has yielded many insights into the processes underlying the development of atherosclerosis. Steinberg et al. (1) were among the first to postulate that modified low-density lipoprotein (LDL) could account for the accumulation of lipid within macrophages, a critical early step in the formation of the atherosclerotic plaque. In the early phases, native LDL may amass in the subendothelial arterial space and may be minimally oxidized by resident vascular cells through the activity of such enzymes as 12/15-lipoxygenase (2). In turn, this minimally modified LDL leads to the production of chemotactic factors and granulocyte and macrophage colony-stimulating factors, which enhance recruitment of circulating monocytes and their differentiation to macrophages in the vessel wall. Plaque stability is believed to be influenced by levels of inflammatory mediators locally, which may stimulate expression of a number of proteolytic enzymes that lead to plaque fragility and rupture. These inflammatory actions encourage further oxidation of LDL, leading to both structural and functional changes in the vessel.

Macrophages avidly accumulate LDL particles modified by oxidation or acetylation through a number of scavenger receptors, including CD36 and scavenger receptors A-I/II, leading to the accumulation of foam cells and development of the atherosclerotic plaque (3,4). At the same time, oxidized LDL species are directly toxic to vascular cells, and lead to endothelial injury and dysfunction, disabling, among other things, the intrinsic antiplatelet effects of this protective barrier, as well as the generation of nitric oxide, with deleterious effects on vascular tone and reactivity (2). The importance of oxidized LDL in atherogenesis has been

further confirmed by the use of specific antibodies to oxidized LDL, which have been shown to localize to atherosclerotic lesions in the vessel wall (5). Furthermore, patients with atherosclerotic disease have elevated levels of circulating antibodies to modified LDL (6,7).

Up until now, investigations have focused on three main dietary antioxidant vitamins (beta-carotene, vitamin C, and vitamin E) and one synthetic antioxidant (probucol). In hypercholesterolemic New Zealand white rabbits, Bocan et al. (8) have demonstrated that antioxidant treatment can lower cholesteryl ester content in thoracic aortic lesions induced by an atherogenic diet, but has no effect on more complicated injury-induced iliac-femoral lesions. These data harmonize with those of earlier studies in the Watanabe heritable hyperlipidemic rabbit that showed slowed progression with probucol (9,10).

In the Antioxidant Supplementation in Atherosclerosis Prevention (ASAP) study, 520 nonsmoking and smoking men and women were randomized to receive either 91 mg (136 IU) of d-alpha-tocopherol, 250 mg of slow-release vitamin C, a combination of these, or placebo for three years (11). The participants were age 45 to 69 years with serum cholesterol ≥ 193.5 mg/dl (5.0 mmol/l). The primary end point was atherosclerotic progression defined as the linear regression slope of the carotid mean intima-media thickness assessed by ultrasonography. The combination regimen was associated with significantly less progression in treated men (mean increase in intima-media thickness of 0.020 mm/year in the placebo group vs. 0.011 in the vitamin combination group, $p = 0.008$); no significant benefit was observed in treated women. The authors concluded that combined supplementation of vitamins E and C in reasonable doses slowed common carotid atherosclerosis.

Despite a plausible biological role for lipoprotein oxidation in disease progression and some evidence of angiographic benefit, the clinical cardiovascular effects of antioxidant treatments have not been satisfactorily demonstrated

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Abbreviations and Acronyms

ASAP	= Antioxidant Supplementation in Atherosclerosis Prevention study
ATBC	= Alpha-Tocopherol-Beta-Carotene cancer prevention study
CAD	= coronary artery disease
CHAOS	= Cambridge Heart AntiOxidant Study
HATS	= High-density lipoprotein Atherosclerosis Treatment Study
HDL-C	= high-density lipoprotein cholesterol
LDL	= low-density lipoprotein
LDL-C	= low-density lipoprotein cholesterol
MI	= myocardial infarction
MVP	= MultiVitamins and Probulcol study
SPACE	= Secondary Prevention with Antioxidants of Cardiovascular disease in End-stage renal disease study

(12). What may account for these disappointing results, and how does the future of antioxidant therapy look in light of the growing predominance of other cardiovascular risk reduction strategies with better results, such as the 3-hydroxy-3-methylglutaryl coenzyme A inhibitors, or statins? This review will evaluate briefly the evidence in favor of and against antioxidant treatment with current agents and discuss these in the context of the statin trials.

ANTIOXIDANT THERAPY IN CORONARY ARTERY DISEASE (CAD) PREVENTION

Observational data. Observational studies have looked at the relationship between antioxidant vitamin intake and cardiovascular events, generally using assessments of intakes based upon dietary recall. In the Nurses' Health study, which included over 87,000 participants (13), coronary events and vitamin E intake were inversely correlated. On the other hand, among almost 40,000 male participants in the Health Professionals' Follow-up study (14), while beta-carotene intake was associated with reduced coronary risk in the whole cohort, vitamin E was associated with reduced risk only in the subgroup who were smokers. Among almost 12,000 subjects in the second National Health and Nutritional Examination Survey (15), there was an inverse relation between vitamin C intake and coronary risk. Finally, in a study in over 11,000 elderly patients (16), fewer coronary events were seen in subjects consuming dietary supplements of vitamin E than in those without supplementation. A Dutch study of 4,802 participants, age 55 to 95 years, with no history of myocardial infarction (MI) reported that the greatest risk for MI was associated with the lowest intake of beta-carotene; no association between vitamins E and C with MI was found (17).

Interventional data. Interventional trials have examined antioxidant vitamin therapy compared with placebo in a randomized, double-blind fashion, in subjects at varying degrees of cardiovascular risk. The large Finnish Alpha-Tocopherol-Beta-Carotene (ATBC) cancer prevention

study, although designed to study the effects of vitamin E and beta-carotene on lung cancer risk in male smokers age 50 to 69 years, also evaluated effects on cardiovascular risk; no cardiovascular benefit was found in the overall cohort. An analysis excluding the 1,862 men with a reported history of MI in ATBC affirmed this negative finding: a small dose of vitamin E in primary prevention had only a marginal and statistically insignificant effect on fatal CAD events and none on nonfatal MI (18). In the Physicians' Health study, among 22,000 U.S. physicians (19), there was no effect of beta-carotene use on coronary events.

The potential benefits of probucol in atherosclerosis have been evaluated both in animal models and in clinical trials that have not made a consistent case in favor of this synthetic antioxidant. In the Probulcol Quantitative Regression Swedish trial, 303 hypercholesterolemic patients with femoral atherosclerosis were treated for three years with probucol (0.5 g twice a day) added to diet plus cholestyramine (8 g twice a day), to see whether probucol could retard progression or induce regression of atherosclerosis, as assessed by change in lumen volume on quantitative angiography in a 20-cm segment of the femoral artery. Although probucol lowered low-density lipoprotein cholesterol (LDL-C) by 12%, no statistically significant benefit on atherosclerotic progression was observed, a null finding perhaps related to the significant reductions in protective high-density lipoprotein cholesterol (HDL-C) induced by probucol (20).

More recently, some groups have studied the effect of probucol and antioxidant vitamins on restenosis after angioplasty. In the MultiVitamins and Probulcol (MVP) study, 317 patients were randomized to receive one of the following: placebo, probucol (500 mg), multivitamin cocktail (beta-carotene, 30,000 IU; vitamin C, 500 mg; vitamin E, 700 IU), or both probucol and multivitamins given twice daily (21). Probulcol monotherapy was associated with a restenosis rate per segment of 20.7%, while the rate in those treated with the combined regimen was 28.9% and in those on placebo 38.9% ($p = 0.009$ for probucol vs. no probucol). Probulcol appeared to exert its antirestenotic effects by improving vascular remodeling after angioplasty (22). Given the size of both trials, no definitive conclusion about probucol's effects on cardiovascular event rates can be made.

Other trials have reported positive results. The Cambridge Heart AntiOxidant Study (CHAOS), conducted in the United Kingdom in 2,000 men and women with angiographic evidence of CAD, showed a benefit of naturally occurring antioxidant therapy (23). Participants were randomized to either placebo or vitamin E at a substantial dose of 400 to 800 IU per day. After a median follow-up period of 17 months, there was a 47% reduction in cardiovascular death and nonfatal MI and a 77% reduction in nonfatal MI among the patients on the antioxidant.

The Secondary Prevention with Antioxidants of Cardiovascular disease in End-stage renal disease (SPACE) study suggested that vitamin E, 800 IU/day, delivered as natural

Table 1. Selected Clinical Trials of Natural Antioxidant Therapies on Atherosclerotic Events*

Trial	N	Antioxidant Dosage	Rx Event† Rate	Placebo Event† Rate	ΔRR
Vitamin E					
ATBC‡	27,271	50 mg (synthetic)	7.6%	7.8%	–4%
CHAOS	2,002	400 or 800 IU (natural)	4.0%	6.6%	–47%
SPACE§	196	800 IU (natural)	5.2%	17.2%	–70%
GISSI	11,324	300 mg (synthetic)	8.0%	8.0%	0
HOPE§	9,541	400 IU (natural)	11.2%	11.0%	+2%
Beta-Carotene					
ATBC	27,271	20 mg (synthetic)	8.0%	7.8%	+1%
Vitamin Cocktails					
ATBC	27,271	50 mg vit. E, 20 mg beta-carotene	7.5%	7.8%	–4%
Heart Protection Study	20,536	600 mg vit. E, 250 mg vit. C, 20 mg beta-carotene	10.4%	10.2%	+2%

*Defined as fatal cardiovascular or coronary artery disease or nonfatal myocardial infarction; †crude event rate as calculated by the number of events divided by the size of the treatment group; ‡primary prevention analysis, excluding 1,862 men with a history of myocardial infarction; §myocardial infarction only. Bold highlighting of trial name denotes statistically significant change in relative risk ($p < 0.05$).

ATBC = Alpha-Tocopherol-Beta-Carotene Cancer Prevention study; CHAOS = Cambridge Heart AntiOxidant study; GISSI = Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico-prevenzione study; HATS = High-density lipoprotein Atherosclerosis Treatment Study; HOPE = Heart Outcomes Protection Evaluation trial; Rx = treated; SPACE = Secondary Prevention with Antioxidants of Cardiovascular disease in End-stage renal disease; vit. = vitamin; ΔRR = change in relative risk.

alpha-tocopherol, decreased the relative risk for a composite primary end point of MI, ischemic stroke, peripheral vascular disease, and unstable angina by 54% ($p = 0.014$) (24). The study was conducted in a small sample ($N = 196$) of patients on hemodialysis with a history of cardiovascular disease, an exceptionally high-risk group, followed for a median 519 days. The annual placebo event rate for definite fatal and nonfatal MI in the SPACE trial was 12.3% per year (17.2% over 519 days), much higher than that in CHAOS (Table 1). These findings complement those from a double-blind prospective study of 40 cardiac transplant patients treated for one year either with placebo or with a cocktail of vitamin C, 500 mg, plus vitamin E, 400 IU, each given twice daily (25). In these transplant patients, for whom accelerated atherogenesis is a common risk, those who had antioxidant treatment had less coronary progression compared with those who did not receive the vitamins. The change in the intimal index was 0.8% versus 8%, respectively ($p = 0.008$). Therefore, additional clinical trials are needed to clarify whether the baseline risk of the patient and the type and dosage of antioxidant used are key determinants of the efficacy of antioxidant therapy.

ANTIOXIDANT PROPERTIES OF STATIN THERAPY

The equivocal interventional data with antioxidant treatment are in sharp contrast with the clinical experience with lipid modification, in which several large, prospective trials have reported significant event reductions. Based on six landmark clinical trials, the statins are widely recognized to reduce the risks for CAD, and to improve survival and decrease recurrence of clinical cardiovascular events in CAD patients (26–32). Their primary mechanism of benefit

probably relates to their ability to lower levels of LDL-C by enhancing hepatic receptor-mediated clearance of apolipoprotein-B-containing particles. However, a number of secondary mechanisms of benefit have been postulated, and some of these have been observed experimentally. These so-called pleiotropic effects include direct anti-inflammatory actions, nitric-oxide-mediated improvement in vascular function, inhibition of cell proliferation, “stabilization” of atherosclerotic plaques, and antioxidant effects (33).

The capacity of statins to prevent lipoprotein oxidation is relevant to this discussion, although the proportion of the clinical benefits associated with these drugs due to this effect remains unknown. In vitro human and animal data suggest that fluvastatin may exert antioxidant effects that protect against lipid peroxidation (34,35). Simvastatin inhibits LDL oxidation by activated human monocyte-derived macrophages, and the metabolites of atorvastatin have been shown to have potent antioxidant effects (36,37). A recent study reported that neonatal rat cardiac myocytes incubated with simvastatin and treated with angiotensin II displayed reduced evidence of hypertrophy compared with myocytes that were not incubated with simvastatin. This effect may be associated with an antioxidant mechanism involving statin-related inhibition of Rac1, a small G protein of the Rho family (38).

TRIALS OF THERAPIES IN COMBINATION WITH ANTIOXIDANTS

The Heart Outcomes Protection Evaluation (HOPE) trial compared ramipril versus placebo in subjects who were at elevated risk for heart disease, but who were without an established indication for angiotensin-converting enzyme

inhibitor therapy (39). There were also groups randomized to vitamin E alone, and to vitamin E plus ramipril. No benefit of vitamin E was seen in either group (40).

The Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico prevenzione trial (GISSI) evaluated the effect of 3.5 years of treatment with n-3 polyunsaturated fatty acids (fish oil supplement, 1 g daily) and vitamin E (200 mg/day), either alone or in combination, versus placebo, in the management of 11,324 recent survivors of MI (41). While fish oil supplementation reduced the relative cardiovascular risk by 10% with only minimal lipid changes, vitamin E produced no benefit. The combination of the two did not lessen the benefit compared with fish oil alone, suggesting that vitamin E did not mitigate the effect.

In contrast, the High-density lipoprotein Atherosclerosis Treatment Study (HATS) (42), a joint U.S.-Canadian trial that investigated the effects of simvastatin, niacin, and antioxidant vitamins, suggested a potential dilution of benefit. The HATS included 160 patients with angiographically demonstrated CAD, normal LDL-C levels, and low HDL-C levels (≤ 35 mg/dl [0.9 mmol/l]). The primary angiographic end point was quantitative change at the end of 36 months of therapy in a 2×2 comparison of: 1) simvastatin (10 to 20 mg)/niacin (2 to 4 g); 2) that regimen + antioxidant vitamins; 3) antioxidant vitamins alone; and 4) placebo. The antioxidant cocktail used included: 800 IU of vitamin E, as d-alpha-tocopherol; 1,000 mg of vitamin C; 100 μ g of selenium; and 25 mg of natural beta-carotene. The average stenosis progressed by 3.9% in the placebo group versus 0.7% in the group receiving both the cocktail and niacin/simvastatin ($p = 0.004$ vs. placebo), while regression of 0.4% was seen in the group treated with simvastatin/niacin without antioxidants ($p < 0.001$ vs. placebo). Therefore, patients who got both therapies appeared to experience less angiographic benefit than those who received simvastatin/niacin alone ($p = 0.02$). Given the small size of this study, additional investigations will be needed before a more serious caution against their combined use is warranted.

The Heart Protection Study evaluated the effects of statin therapy and antioxidant vitamins in 20,536 patients at high risk of coronary events with a wide range of baseline serum cholesterol levels (31,32). Subjects in the trial were at high risk for an ischemic event, based upon existing coronary disease, other vascular disease, diabetes, or hypertension, and were treated for a median of five years with simvastatin 40 mg/day or placebo, along with, in a 2×2 design, an encapsulated antioxidant "cocktail" (vitamin E, 600 mg; vitamin C, 250 mg; beta-carotene, 20 mg), or matching placebo. Although simvastatin significantly reduced the risks for coronary morbidity and mortality, there was no evidence of either benefit or harm from antioxidant monotherapy, nor did concomitant antioxidants mitigate the benefits of statin therapy.

DISCUSSION

While the use of antioxidants to prevent CAD has not been satisfactorily demonstrated, Steinberg and Witztum (43) have recently argued persuasively in favor of continued research, citing a number of limitations and unanswered questions related to earlier attempts to evaluate the oxidation hypothesis in clinical trials. For example, because it is not certain exactly where and how LDL gets oxidized *in vivo*, pharmacokinetic differences between available antioxidants may be especially germane to the findings seen thus far (e.g., beta-carotene given in large doses does not appear to protect LDL from oxidation *ex vivo*; vitamin C is transported in the aqueous phase, while vitamin E takes up residence in lipoproteins) (12). Other reasons may explain why antioxidant intervention trials have not been so successful in humans. The agents studied may not be potent enough, and the doses used may be too low. What kinds of potent novel antioxidants will need to be developed?

The patients studied in trials have had advanced degrees of cardiovascular disease; prevention of oxidative modification may be more important in earlier stages of lesion development, but less effective for established atherosclerosis. The rate of oxidation in humans also may be less than in other experimental animal models, accounting for the discordance between animal and human trials. The lack of benefit in the high-risk Heart Protection Study cohort suggests that high risk alone cannot be the basis for initiating antioxidants (32). As seen in the ASAP study (11), the MVP study (21), the SPACE study (24), and Fang et al. (25), the patient profile may be an important consideration in the efficacy of antioxidant therapy, especially in those groups for whom the mechanical processes underlying atherosclerosis may be accelerated. Aggressive antioxidant supplementation may prove a specialized approach for cardiovascular prevention in certain kinds of patients.

A small study showed that pretreatment with vitamin C (1 g) and vitamin E (800 IU) appeared to inhibit postprandial endothelial dysfunction after a high-fat meal, as assessed by brachial ultrasonography (44). There is some controversy whether antioxidants taken as diet supplements yield qualitative differences from those consumed in a diet that includes antioxidant-rich foods. In a combined analysis from the Nurses' Health study and the Health Professionals' Follow-up study, every one-serving/day increase in consumption of leafy green vegetables and vitamin C-rich fruits and vegetables was associated with a 4% decrease in relative risk for coronary disease (45). Dietary plant-derived flavonoids, as found in grape juice, wine, and soy products, have also gained attention for their antioxidant properties, based on observational trials that associate intake of these substances with cardiovascular protection (46). The prospective single-blinded Lyon Diet Heart study reported a 72% relative risk reduction in recurrent coronary events ($p = 0.0001$) in heart attack survivors on a Mediterranean diet compared with a prudent "Western"-type diet (47). The

composition of the Mediterranean diet favors foods with antioxidant potential, but the overall diet includes other cardioprotective characteristics, such as reduced saturated fats, greater use of unsaturated fats like olive oil, and fish and plants high in concentrations of alpha-linolenic acid (48). It may be the synergy of this overall more healthful eating pattern that accounts for benefits observed than any individual effect of the diet.

In patients with coronary disease, lipid-altering therapy with statins should be a cornerstone of pharmacologic treatment, with a priority of getting LDL-C levels to recommended targets. Both groups receiving simvastatin in HATS had mean final LDL-C levels below 80 mg/dl (2.07 mmol/l), and the treatments were well-tolerated. Simvastatin/niacin therapy was associated with a mean increase in HDL-C of about 30%, suggesting that combined therapy may be appropriate when HDL-C levels are low.

In conclusion, we cannot discount the oxidation hypothesis in human atherosclerosis, and experiments to date have only illustrated the difficulties in testing this complex issue. While the search continues for insights and potential therapies related to lipoprotein oxidation, better use of the therapies already available is needed to address unfavorable risk factor profiles and to reduce cardiovascular risk.

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